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Effect of triazophos on mitotic activity and chromosomal behavior in root meristems of *Allium cepa* L.

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Abstract

Effect of triazophos (an organophosphorous insecticide) on mitotic activity and chromosomal behavior in the meristematic region of root tip cells of *Allium cepa* L. was assessed. The insecticide showed mitotic depression and positive chromo-toxic effects. Abnormalities, such as stickiness, plasmolysed cells, equatorial plate shifting, polar shifting, irregular chromosome arrangement, precocious arms formation, bridge formation, C-metaphase, fragmentation of chromosomes, unequal cytokinesis, diagonal cytokinesis, delayed cytokinesis and formation of binucleated cells, were recorded in the chemically pretreated root meristem.

Key-words: chromosomal and cellular abnormalities, cytotoxic effect, mitotic index, phase indices.

Introduction

A large number of agrochemicals, such as pesticides or insecticides, are being used in different parts of the world in order to protect agricultural and horticultural crops from diseases and pests. Increased utilization of pesticides in modern agriculture has raised the issues of their negative effects on the environment and human health. Although the use of pesticides has become a necessity, the frequent and indiscriminate use of these chemicals proved to have many undesirable secondary consequences on plants (Epstein and Legator 1971; Amer and Farah 1974). In many developing countries, including Nepal, insecticides are used in higher concentrations than their recommended dose. Increase use of such chemicals is of great concern and warrants testing of their cytotoxic or genotoxic effect. Triazophos is a non-systemic organophosphorous insecticide. It has strong permeability to plant tissue. It is chiefly used for controlling insects (Lepidoptera) that damage agricultural, horticultural and forest crops. The main fields of application are cotton, sugarcane, maize, potatoes, vegetables, fruits, coffee and ornamentals. The present study has been undertaken to determine the effect of triazophos on mitotic activity and chromosomal behavior in root meristems of onion (*Allium cepa* L.).

Materials and Methods

Root meristems of *Allium cepa* were treated with different concentrations of triazophos (0, 25, 50, 75 and 100%) for different periods (3, 6, 12, 24 and 48 h). The treated and controlled roots were fixed in acetic acid and alcohol (1:3 v/v) at 10:00 am and cytological preparations were made using the acetocarmine squash technique. Permanent slides were prepared by using Celarier's method (Celarier 1956). Observations were recorded on around 4000 cells. Mitotic and phase indices, and abnormalities were scored and analyzed following Levan's method (cf. Kihlman 1971; Medeiros and Takahashi 1987). Chi-square test was performed to assess the inhibitory effect of triazophos on mitotic index (MI) in relation to time of treatment.

Results

EFFECTS ON MITOTIC INDEX

The mitotic index (MI) value decreased gradually with the increase in triazophos concentration and duration of treatment (Fig. 1). The control value of MI was 32.58, and this value was dropped from 31.96 to 29.61 (at 3 h after treatment), 32.43 to 26.35 (6 h after treatment), 27.52 to 24.48 (12 h after treatment), 25.80 to 22.27 (24 h after treatment) and 19.09 to 13.33 (48 h after treatment) in 25, 50, 75 and 100% concentrations of triazophos, respectively. In all treatments, the decreased MI value with increasing treatment hour is attributed to mitotic inhibition.

EFFECTS ON PHASE INDICES

The control value of prophase index was 78.96%. The highest value of prophase index (prophase index = 87.52%) was observed in 75% triazophos at 48 h after treatment and the lowest value (prophase index = 70.61%) was recorded in 100% triazophos at 24 h after treatment (Fig. 2).

The control value of metaphase index was 10.93%. The highest value of metaphase index (15.94%) was observed in 100% concentration of triazophos at 24 h after treatment (Fig. 3). The metaphase index generally increased with the increase in concentration of the insecticide and duration of treatment. As compared to control (0% triazophos), the metaphase index was higher in all other concentrations and duration of treatment, except in 25% and 50% concentrations for 48 h after treatment and in 75% concentration for 3 h and 6 h after treatments.

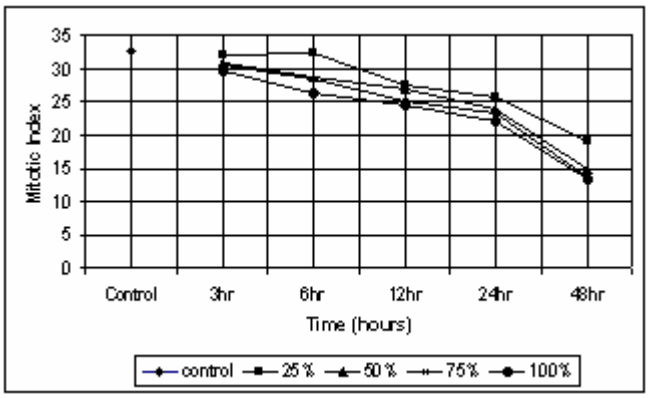

Fig. **1**. Mitotic index (MI) of *Allium cepa* root tip cells at different concentrations of triazophos and duration of treatment.

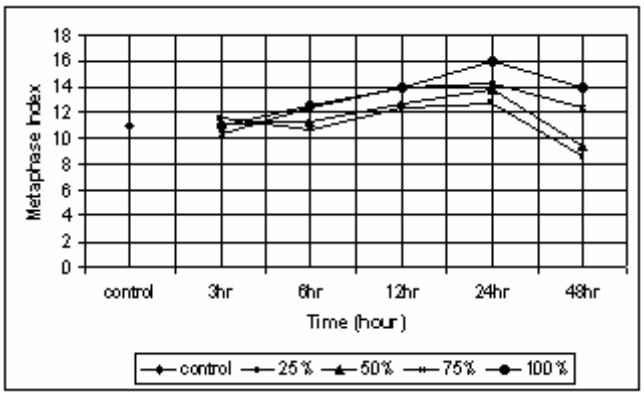

Fig. **3**. Metaphase index of *Allium cepa* root tip cells at different concentrations of triazophos and duration of treatment.

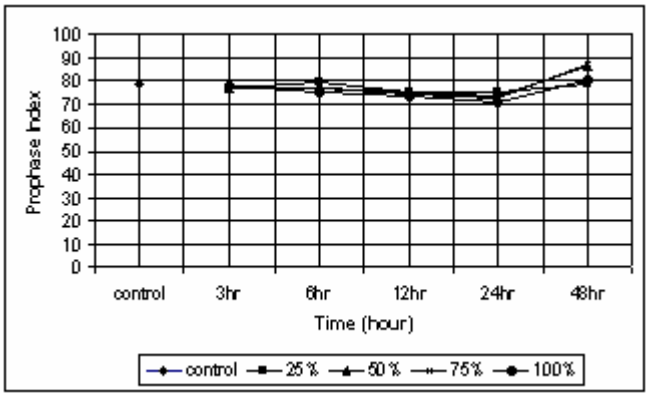

Fig. **2**. Prophase index of *Allium cepa* root tip cells at different concentrations of triazophos and duration of treatment.

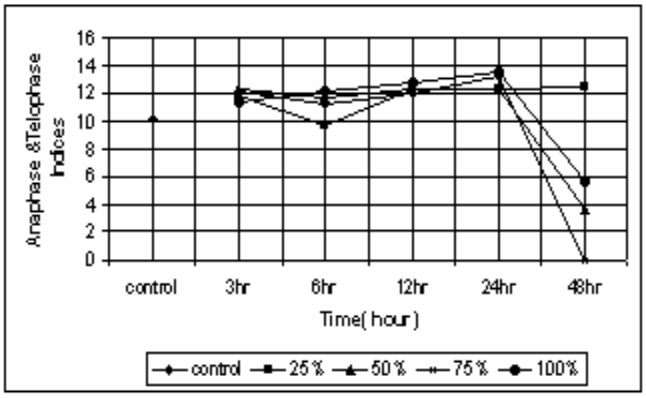

Fig. **4**. Anaphase-telophase index of *Allium cepa* root tip cells at different concentrations of triazophos and duration of treatment.

The value of ana- and telophase index was also increased with the increasing triazophos concentration and duration of treatment until 24 h (Fig. 4). The control value of anaphase index was 10.09%. The highest value of ana-telophase index (13.43%) was observed in 100% triazophos at 24 h after treatment. The value of ana-telophase index was higher than the control value (0% triazophos) in all concentrations and duration of treatment, except at 48 h of treatment in 50% and 100% concentrations, and 6 h of treatment in 25% concentration of triazophos. The value was almost zero in 75% concentration at 48 h of treatment.

EFFECTS ON CHROMOSOMAL BEHAVIOR

Triazophos was capable of inducing various types of chromosomal abnormalities in root tip cells of *Allium cepa* during mitotic cell division (Fig. 5). Disturbed prophase, clumping of chromosomes, fragmented chromosomes, binucleated prophase and unequal condensation of chromatins were the abnormalities found in prophase. Similarly, equatorial plate shifting (diagonally placed equatorial plate was the most common type of abnormality), C-metaphase, disturbed metaphase, fragmented metaphase and stickiness in metaphasic chromosomes were the abnormalities found during metaphase stage. The common abnormalities observed during anaphase stage were polar shifting (diagonal anaphase), precocious arms, unequal rate of movement of chromosome, and laggard and bridge formation. Clumped and sticky anaphase was also observed. The abnormalities during telophase stage were polar shifting, unequal condensation of nuclei, unequal cytokinesis, diagonal cytokinesis, and delayed cytokinesis forming binucleated cells.

Discussion

Triazophos was capable of inducing various types of abnormalities in root meristem of *Allium cepa*. The analytical data of mitotic and different phase indices were used for positive or negative effect in cell as well as mitotic cycle on experimental basis. The mitotic index value was decreased when the meristems were treated with increasing concentration of the insecticide and duration of treatment, thus suggesting inhibitory effects of the insecticide on mitotic cell division. The inhibition in the mitotic index value indicates that the suspension of the insecticide interferes with the normal sequence of cell division. Mitotic inhibition by pesticides is remarkably associated with the blocking of mitotic cycle during interphase, which may results from a prolonged G2 period or to the inhibition of DNA and RNA biosynthesis (Mohandas and Grant 1972). Increase concentration and prolonged period of treatment resulted in increased reductions in the amounts of both nucleic

acids (Mohandas and Grant 1972; Badr and Ibrahim 1987). In the present study, Chi-square test showed that the inhibitory effect of triazophos on mitotic index (MI) in relation to time of treatment was statistically significant (observed value χ^2 =15.4, tabulated value χ^2 = 9.48). This shows the effectiveness of the insecticide related with the time of treatment in cell division. Similar results were observed by Medeiros and Takahashi (1987).

The increasing value of prophase index indicates prophase poisoning as a result of the triazophos treatment. This could be due to the elongation of prophase stage, which indicates the effect of pesticide on spindle formation. Similar effects were observed by Kaul (1972) in amide treated root tips and by Shehab (1980) in alcohol treated root tips of onion. The increasing percentage of abnormalities in metaphase and ana-telophase than in prophase may be due to decreasing percentage of prophase. The increase in metaphase index could be explained on the basis of failure of abnormal behavior of spindle mechanism (Kabarity and Mallah 1980). The increasing ana- and telophase index is due to delay in the completion of mitotic cycle. Besides these abnormalities in dividing cells, some abnormalities were also observed in interphasic cells. Among them, shifting of nucleus to the polar position, plasmolysed cells, tri- or tetra-nucleoli stage etc. were common. The shifting of nucleus to the polar position is due to the imbalance in the osmoregulation of the cells that caused cells to be plasmolysed and shifted the nucleus aside sometimes touching the cell wall (Rangaswamy *et al*. 1981).

Above results strongly supports the cytotoxic nature of triazophos. The decrease in mitotic index of cells treated with triazophos suggests its inhibitory effect in cell division. It is capable of inducing various chromosomal abnormalities, the degree of which increases with the concentration and duration of the treatment. The abnormalities, like fragmentation of chromosomes, breaks and bridges, suggest clastogenic effect of triazophos. Highly stickiness and disturbed prophase also suggest its cytotoxic effect. Presence of C-metaphase, laggards, precocious chromosomes and binucleate cells suggest that the chemical affects the mitotic spindles by its turbogenic character. Thus, the chemical triazophos has both inhibitory and mutagenic effects and should be used carefully.

Acknowledgements

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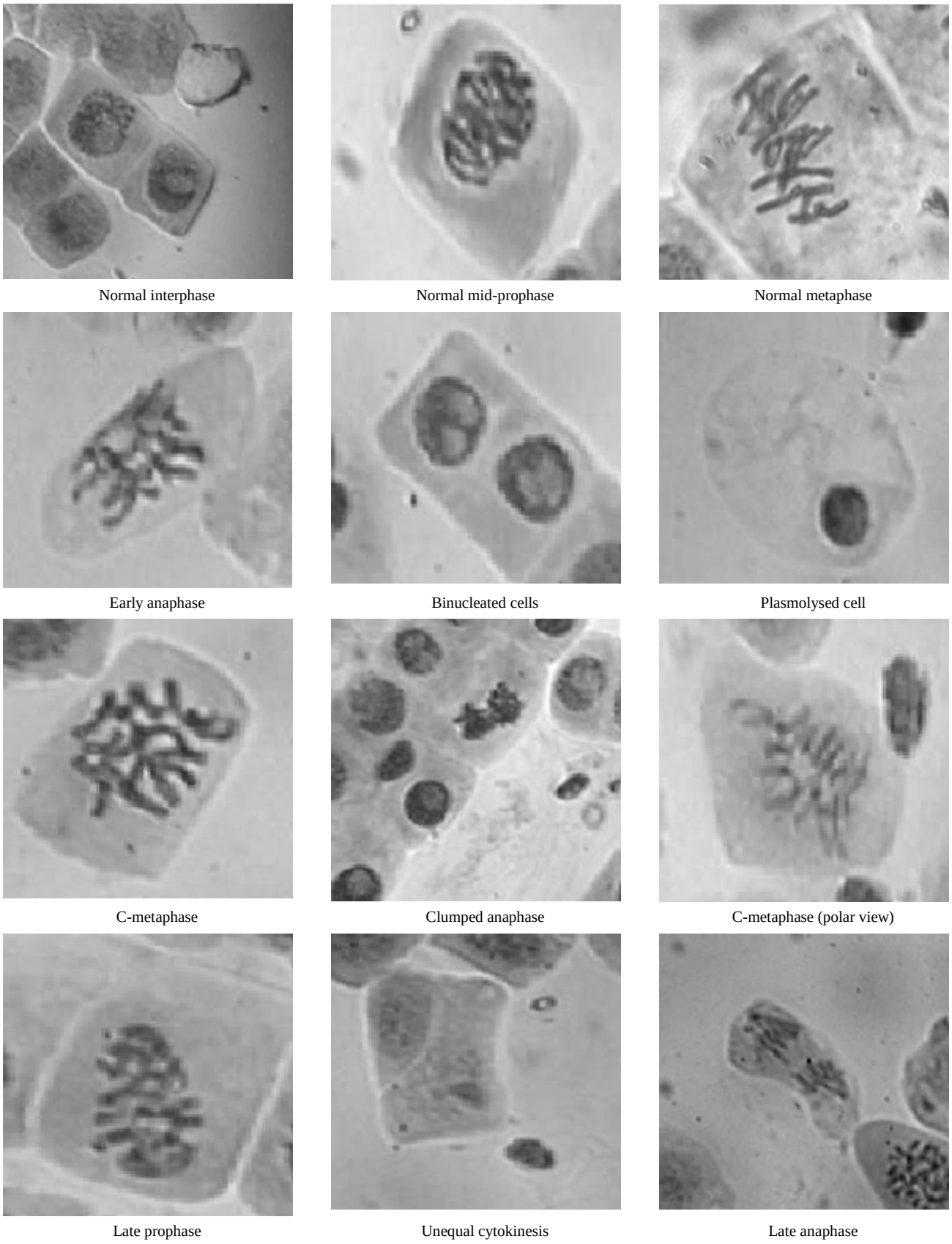

Fig. 5. Chromosomal abnormalities induced by triazophos.